bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Restriction enzyme accessibility assay

Sharon E Torigoe

Updated date: Dec 29, 2020

An abbreviated version of this protocol was published in eLIFE in Aug 2013

ATP-dependent chromatin assembly is functionally distinct from chromatin remodeling

DOI: 10.7554/eLife.00863

Detailed protocol

Restriction Enzyme Accessibility (REA) assays are used to evaluate for chromatin remodeling activity in vitro. These assays work on the premise that nucleosomes block restriction enzymes from cutting DNA. Hence, as chromatin remodeling proceeds in a REA reaction, cut sites for restriction enzymes become more accessible, leading to increased digestion of DNA. This method can be used to measure the activity of purified chromatin remodeling factors and also to evaluate the effects of other chromatin factors on chromatin remodeling.

Reagents:

1. Salt-dialyzed chromatin
    a. Reconstitute nucleosomes onto plasmid DNA by the salt-dialysis method.
    b. It is highly preferred to use "under-assembled" chromatin, in which the mass ratio of core histones to DNA is approximately 0.8, rather than the typical 1.0.
2. Purified chromatin remodeling factors: The concentration to use will depend on the specific proteins and the density of the salt-dialyzed chromatin, so you will need to test a range of concentrations when testing a chromatin remodeling factor for the first time. Chromatin remodeling activity can be observed with 15-30 nM of the chromatin remodeling factors ACF, CHD1 and BRG1.
3. Restriction enzymes: Select a restriction enzyme that has several cut sites on the plasmid DNA used to reconstitute nucleosomes. A very good choice is HaeIII, which has a four nucleotide recognition sequence that is relatively common.
4. Ultrapure or double-distilled water
5. 10X D-Loop Buffer: 250 mM Tris-acetate (pH 7.5), 100 mM Mg-acetate, 10 mM DTT
6. 2 mg/mL BSA
7. 20 mM ATP
8. 2.5 mg/mL Proteinase K
9. Glycogen Stop Buffer: 20 mM EDTA, 0.2 M NaCl, 1% (w/v) SDS, 0.25 mg/mL glycogen
10. Phenol:chloroform:isoamyl alcohol
11. 2.5 M ammonium acetate
12. Ethanol
13. 1X loading buffer: 6.25% (v/v) glycerol, 1X TBE Buffer, with Orange G dye
14. 10X TBE (diluted to 1X TBE for gel electrophoresis)
15. Agarose
16. Ethidium bromide stain

Equipment:

1. Micropipettes
2. Water baths set to 27°C and 37°C
3. Microcentrifuge
4. Vortex mixer
5. Horizontal gel electrophoresis apparatus
6. Electrophoresis power supply
7. Gel imaging system with UV transilluminator

Procedure:

1. Assemble Reactions for REA Assays:
    a. Useful tips:
        i. For the REA reactions, use low-binding microcentrifuge tubes and micropipette tips to reduce loss of proteins and DNA due to adherence to plastic surfaces.

ii. Except for the restriction enzyme, all solutions and reagents should be brought to room temperature prior to pipetting.
iii. Mix reaction reagents by flicking the tube.
iv. It is common practice to perform the following control reactions: (1) salt-dialyzed chromatin in the absence of purified chromatin remodeling factors; (2) bare DNA (200 ng of the same plasmid DNA used to reconstitute nucleosomes) in the absence of purified chromatin remodeling factor.

b. Each REA reaction is 50 µL, containing the following:
- 5 µL of 10X D-Loop Buffer
- 2.5 µL of 2 mg/mL BSA
- 5 µL 20 mM ATP
- 200 ng of salt-dialyzed chromatin (quantification is based on the amount of DNA)
- Purified chromatin remodeling factor
- 10 units of restriction enzyme
- Ultrapure or double-distilled water to 50 µL

c. For each REA reaction, mix together the following (see volumes above) in a low-binding microcentrifuge tube: ultrapure water, D-Loop Buffer, BSA, and ATP. Flick the tube to mix.
d. Then add, in the following order: salt-dialyzed chromatin, purified chromatin remodeling factor, and restriction enzyme. Flick the tube to mix after each.
e. Incubate the REA reactions for 1 hour in a water bath set at 27°C.

2. Deproteinate Samples:
a. To each reaction, add 5 µL of 2.5 mg/mL Proteinase K and 100 µL of Glycogen Stop Buffer.
b. Invert tubes several times to mix, and incubate for 30 minutes in a water bath set at 37°C.

3. Extract DNA:
a. To each reaction, add 150 µL of phenol:chloroform:isoamyl alcohol.
b. Vortex vigorously for 30 seconds.
c. Centrifuge for 5 minutes, at >16,000 x *g* (maximum speed in most microcentrifuges), at room temperature.
d. Carefully pipette 145 µL of the upper aqueous phase and transfer to a new microcentrifuge tube. Do not use tubes made with low-binding plastic.
e. To each aqueous phase, add 25 µL of 2.5 M ammonium acetate and 600 µL of 100% ethanol.
f. Invert tubes several times to mix.
g. Centrifuge for 20 minutes, at >16,000 x *g*, at room temperature.
h. Carefully remove most of the supernatant using a P1000 micropipette. The DNA will be small pellet near the bottom of the tube, so be careful not to dislodge it.
i. Briefly centrifuge the tubes again to collect remaining liquid. Remove using a P200 and/or P10 micropipette.
j. Air-dry the DNA pellet for 5-10 minutes.

4. Analyze Digested DNA:
a. Resuspend the DNA pellet in 5 µL 1X loading buffer.
b. Subject the DNA to electrophoresis on a 1.5% agarose gel, in 1X TBE, until the Orange G dye reaches the bottom of the gel.
c. Visualize the DNA by staining with ethidium bromide after electrophoresis. Incubate the gel in ethidium bromide stain for 30 minutes, with gentle agitation. Wash away excess stain with water (typically, two rinses in water, 10 minutes each).
d. Image the gel using a UV transilluminator.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Torigoe, S. (2020). Restriction enzyme accessibility assay. Bio-protocol Preprint. bio-protocol.org/prep725.
2. Torigoe, S. E., Patel, A., Khuong, M. T., Bowman, G. D. and Kadonaga, J. T.(2013). ATP-dependent chromatin assembly is functionally distinct from chromatin remodeling. eLIFE. DOI: 10.7554/eLife.00863